IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	COHEN-HAGUENAUER	Examiner:	E. SORBELLO
Serial No.:	09/433,322	Group Art Unit:	1633
Filed:	NOVEMBER 3, 1999	Docket No.:	8076.110USC2
Title:	RETROVIRAL VECTOR FOR THE TRANSFER AND EXPRESSION OF GENES FOR THERAPEUTIC PURPOSES IN EUKARYOTIC CELLS		

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By: _____
Name Chris Stordahl

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Please kindly amend the above-referenced application for patent as follows. Applicants respectfully request entry of this Preliminary Amendment.

In the Specification

Please add the paragraph beginning at page 1 after the title with the following paragraph:

--Cross Reference to Related Application

This application is a Continuation application of U.S. Serial No. 09/433,322, filed 11/3/99, which is a continuation of 08/270,662 filed June 30, 1994, now abandoned--

Please replace the paragraph beginning at page 3, line 8, with the following rewritten paragraph:

--According to a first embodiment, the object of the invention is a recombinant retroviral vector for the cloning and/or express and/or transfer of an exogenous nucleotide sequence, characterized in that it consists of any sequence contained in the ClaI-PvuII fragment situated approximately between nucleotides 7702 and 1527 (SEQ ID NO: 11) of the sequence given in Figure 1 and comprising the LTR sequence included between nucleotides 7842 and 144, the PBS site starting at nucleotide 145, the packaging sequence included in the sequence of 250 nucleotides following the end of the LTR sequence, the said sequence being capable of controlling the cloning and/or expression and/or transfer of the exogenous sequence.--

Please replace the paragraph beginning at page 3, line 19, with the following rewritten paragraph:

--According to another embodiment of the invention, the recombinant vector is characterized in that it consists of any sequence contained in the ClaI-BamHI fragment comprising the nucleotides 7702 to 310 (SEQ ID NO: 13) of the sequence shown in Figure 1, and comprising the LTR sequence included between the nucleotides 7842 and 144, the PBS site starting at nucleotide 145, the packaging sequence included in the sequence of 250 nucleotides following the end of the LTR sequence, the said sequence being capable of controlling the cloning and/or expression and/or transfer of the exogenous sequence, whatever its transcriptional orientation with respect to the transcriptional orientation of the virus.--

Please replace the paragraph beginning at page 3, line 30, with the following rewritten paragraph:

--According to this second embodiment of the invention, the vector is thus a retroviral vector for the cloning and/or expression and/or transfer of an exogenous nucleotide sequence consisting of any sequence contained in the ClaI-BamHI fragment situated approximately between nucleotides 7702 and 310 (SEQ ID NO: 13) of the sequence given in Figure 1, the said sequence having the capacity to control the cloning and/or expression and/or transfer of the exogenous sequence.--

Please replace the paragraph beginning at page 4, line 17, with the following rewritten paragraph:

--According to an attractive embodiment of the invention, the recombinant vector is characterized in that it consists of all of the ClaI-PuvII fragment, comprising nucleotides 7702 to 1527 (SEQ ID NO: 11) of the sequence shown in Figure 1.--

Please replace the paragraph beginning at page 4, line 21, with the following rewritten paragraph:

--Another preferred retroviral vector consists of all of the ClaI-BamHI fragment (7702 to 310) (SEQ ID NO: 13).--

Please replace the paragraph beginning at page 5, line 16, with the following rewritten paragraph:

--Preferably the recombinant vector additionally comprises a part of the gag sequence situated between the nucleotides 619 and 2235 (SEQ ID NO: 14) of the sequence shown in Figure 5, in particular the sequence included between nucleotides 619 and 1527 (SEQ ID NO: 15) of the sequence shown in Figure 1.--

Please replace the paragraph beginning at page 6, line 21, with the following rewritten paragraph:

--A useful vector of the invention lacking the envelope sequence consisting of the fragment comprising nucleotides 7806 to 1527 (SEQ ID NO: 12) of the sequence shown in Figure 1.--

Please replace the paragraph beginning at page 6, line 24, with the following rewritten paragraph:

--The invention also relates to a recombinant vector such that the sequence contained in the ClaI-PvuII fragment (7702-1527) (SEQ ID NO: 11) and/or this fragment and/or the sequence contained in the fragment ClaI-BamHI (7702-310) (SEQ ID NO: 13) and/or this fragment is replaced either by a sequence hybridizing under conditions of high

stringency with the sequence corresponding to the above-mentioned fragments or by a sequence having an at least 95% nucleotide homology with the sequence corresponding to the above-mentioned fragments or at least 85% homology in the case of the U3 sequence.--

Please replace the paragraph beginning at page 12, line 1, with the following rewritten paragraph:

--Other characteristics and advantages of the invention will become apparent in the examples and Figures which follow.

Figure 1: Sequence of viral DNA used for the construction of the vector FOCH29.

Figure 2: A: Restriction map of the vector FOCH29.

References Seq "FB29"

Cla ----› U3 140 (7702-7842)
U3 ----› R410 (7842-8255)
R ----› CBS 145 (0-145)

PvuII ----› BamHI 208
PvuII ----› PvuII 1098
PvuIIMT ----› PvuII 1669
BsmAI ----› 55/150/765/1766/2531/2684--

Please replace the paragraph beginning at page 15, line 9, with the following rewritten paragraph:

--The oligonucleotide sequences used are:

1°) - for the first pair:

5' CTGCTGACGGGAGAAGAAAAAC-3' (SEQ ID NO: 3)

5' CCCGCTCAGAAGAACTCGTC-3' (SEQ ID NO: 4)

2°) - for the second pair:

5' GACGAGTTCT TCTGAGCGGG-3' (SEQ ID NO: 5)

5' GATCTGAACT TCTCTATTCTTG-3' (SEQ ID NO: 6)--

Please replace the paragraph beginning at page 28, line 5 with the following rewritten paragraph:

--The viral integration was investigated by molecular methods, in particular by PCR using the following primers: oligo-SENSE situated in the beta-galactosidase gene with the following sequence: 5'- CGA CTC CTG G AG CCC GTC AGT ATC-3' (SEQ ID NO: 7); oligo-ANTISENSE situated in the viral LTR, overlapping between R and the start of U5 (LTR-508): 5'- CAG CGA GAC CAC GAG TCG GAT GC-3' (SEQ ID NO: 8) in a region which has been prepared by EspI/BssHII deletion.--

Please replace the paragraph beginning at page 29, line 4 with the following rewritten paragraph:

--The construction was made from the plasmid pUC19 including the EcoRI-PvuII fragment described in part B1-1: enzymatic cutting by the restriction enzyme EspI (or IsoCellI) at position 7864 (namely +23 of the viral LTR). At the 5' end the bases generated by a EcoRI cut were artificially added to a double stranded synethetic oligonucleotide complementary to the 23 bases of the LTR (140 bases, 103 of which are bases of the envelope). At the 3' end the oligonucleotide is complementary to the cohesive SpeI ends. The oligonucleotide sequences are the following: oligo-SENSE 5' - AAT TCA ATG AAA GAC CCC AAA TTG C-3' (SEQ ID NO: 9), oligo-ANTISENSE 5' - TAA GCA ATT C GG TGG GGT CTT TCA TTG-3' (SEQ ID NO: 10).--

Please replace the paragraph beginning at page 30, line 1 with the following rewritten paragraph:

--In this perspective of improving viral safety, an alternative construction was prepared in which the GAG of FB29 is cut at the unique AhaIII (or isoDral) site at position 1031 (SEQ ID NO:16); only a quarter of the GAG sequences are then conserved. The cut generated by AhaIII having blunt ends as for PvuII used for the cloning of FOCH29, the construction was made exactly superposable, the cloning upstream not being modified; downstream, the blunted ended SmaI site of the polylinker of pUC19 was used.--

In the Claims

Please cancel Claims 2-26 without prejudice or disclaimer of the subject matter contained therein.

Please add and consider new claims 27-46.

27. (New) A retroviral vector comprising an isolated nucleotide sequence comprising: a 5' long terminal repeat of Friend Murine Leukemia Virus, a primer binding site of Friend Murine Leukemia virus, a packaging sequence of Friend Murine Leukemia Virus, and a 3' Long terminal repeat of a Friend Murine Leukemia Virus, and at least one exogenous nucleotide sequence encoding a polypeptide operably linked to the isolated nucleotide sequence, wherein the isolated nucleotide sequence provides for transfer and integration of the exogenous nucleotide sequence in a host cell.

28. (New) The retroviral vector of claim 27, wherein the exogenous nucleotide sequence is located between the 5' and the 3' long terminal repeats.

29. (New) The retroviral vector of claim 27, wherein the exogenous nucleotide sequence encodes a selectable marker gene.

30. (New) The retroviral vector of claim 27, wherein the exogenous nucleotide sequence encodes a gene selected from the group of genes encoding HIV retroviral antigens; cytokines, GP170, metallothionine IIA, FACC, beta galactosidase, PLP, tumor necrosis factor, and tissue inhibitor of metalloproteinases (TIMP).

31. (New) The retroviral vector according to claim 27, wherein the at least one exogenous nucleotide sequence comprises an exogenous promoter to provide for expression of the gene.

32. (New) The retroviral vector according to claim 31, wherein the promoter is selected from the group of promoters for epidermal growth factor receptor and the promoter for phosphoglycerate kinase enzyme.

33. (New) The retroviral vector according to claim 27, wherein the host cell is selected from the group of mammalian cell, dog cell and human cell.

34. (New) A self inactivating retroviral vector comprising: an isolated nucleotide sequence comprising a 5' long terminal repeat from Friend Murine Leukemia Virus, a primer binding site from Friend Murine Leukemia Virus, a packaging sequence from Friend Murine Leukemia Virus and a 3' long terminal repeat of Friend Murine Leukemia Virus, wherein the 3' long terminal repeat has a deletion of the nucleotide sequences encoding viral promoter and enhancer sequences, and wherein the 3' long terminal repeat has the nucleotide sequence of the restriction fragment of BamH1-Bgl II of pFOCH29 with a deletion of the nucleotide sequence of restriction fragment EspI-BssHII.

35. (New) A self inactivating retroviral vector comprising: an isolated nucleotide sequence comprising a 5' long terminal repeat from Friend Murine Leukemia Virus, a primer binding site from Friend Murine Leukemia Virus, a packaging sequence from Friend Murine Leukemia Virus, a 3' long terminal repeat of Friend Murine Leukemia Virus, wherein the 3' long terminal repeat has a deletion of the nucleotide sequences encoding viral promoter and enhancer sequences, and wherein the 3' long terminal repeat has the nucleotide sequence of the restriction fragment of BamH1-Bgl II of pFOCH29 with a deletion of the nucleotide sequence of restriction fragment EcoRV-BssHII of pFOCH29.

36. (New) A retroviral vector comprising: an isolated nucleotide sequence comprising a 5' long terminal repeat of Friend Murine Leukemia Virus strain FB29, a primer binding site of Friend Murine Leukemia Virus strain FB29, a packaging sequence of Friend Murine Leukemia Virus strain FB29 and a 3' long terminal repeat of Friend Murine Leukemia Virus strain FB29, wherein the isolated nucleotide sequence lacks a nucleotide sequence encoding a functional envelope protein of Friend Murine Leukemia Virus strain FB29; and wherein the isolated nucleotide sequence is operably linked to at least one exogeous nucleotide sequence encoding a polypeptide, wherein the isolated nucleotide sequence provides for transfer and integration of the at least one exogenous nucleotide sequence in a host cell.

37. (New) The retrovial vector according to claim 36, wherein the vector has a titer of about 10^4 PFU per ml or greater.

38. (New) A host cell transformed with the retroviral vector of claim 27.

39. (New) A host cell transformed with the retroviral vector of claim 36.

40. (New) A host cell transformed with the retroviral vector of claim 35.

41. (New) A host cell transformed with the retroviral vector of claim 34.

42. (New) A process for expression of an exogenous nucleotide sequence encoding a polypeptide in a host cell comprising:

a) growing the host cells in *in vitro* culture;

b) contacting the host cells with the retroviral vector according to claim 27, claim 34, claim 35, or claim 36; and

c) detecting the expression of the exogenous nucleotide sequence encoding a polypeptide in the host cells.

43. (New) The process according to claim 42, further comprising maintaining the transfected cells in culture.

44. (New) A process for expression of an exogenous nucleotide sequence encoding a polypeptide in an animal comprising:

a) isolating and culturing a host cell type from the animal;

b) transfecting the host cell type with the retroviral vector according to claim 27 , claim 34, claim 35, or claim 36;

c) selecting the transfected host cell types expressing the exogenous nucleotide sequence encoding a polypeptide; and

d) reintroducing the transfected host cell types expressing the exogenous nucleotide sequence encoding a polypeptide into the animal.

45. (New) A retroviral vector comprising an isolated nucleotide sequence comprising a 5' long terminal repeat of Friend Murine Leukemia Virus , a primer binding site of Friend Murine Leukemia Virus, a packaging sequence of Friend Murine Leukemia Virus , and a 3' long terminal repeat of Friend Murine Leukemia Virus , wherein the isolated nucleotide sequence lacks a nucleotide sequence encoding a functional envelope protein of Friend Murine Leukemia Virus strain FB29; and wherein said 5' long terminal repeat, primer binding site, and packaging sequence are encoded by the nucleotide sequence of SEQ ID NO. 11, and wherein the 3' long terminal repeat has the nucleotide sequence of the restriction fragment BamHI- Bgl II of pFOCH29 with a deletion of the nucleotide sequence of restriction fragment EspI-BssH11.

46. (New) A retroviral vector comprising an isolated nucleotide sequence comprising a 5' long terminal repeat of Friend Murine Leukemia Virus, a primer binding site of Friend Murine Leukemia Virus, a packaging sequence of Friend Murine Leukemia Virus, and a 3' long terminal repeat of Friend Murine Leukemia Virus, wherein the isolated nucleotide sequence lacks a nucleotide sequence encoding a functional env protein of Friend Murine Leukemia Virus strain FB29, and wherein the 5' long terminal repeat, primer binding site and packaging sequence of Friend Murine Leukemia Virus are

encoded by the nucleotide sequence of SEQ ID NO: 11, and wherein the 3' long terminal repeat has the nucleotide sequence of the restriction fragment of BamHI-Bgl II of pFOCH29 with a deletion of the nucleotide sequence of restriction fragment EcoRV-BssHII.

REMARKS

Applicant has amended the specification and presented new claims 27 through 46 for entry.

Applicant has amended the specification to add the continuation data and to add the sequence identifiers that correspond to the sequence listing. Applicant has also corrected certain typographical errors. At page 5, line 28, the ending nucleotide of the nucleotide sequence encoding the gag gene product is changed from 2245 to 2235. The support for this correction is at Figure 5b, line 8, showing that the end of the gag gene is at the nucleotide position 2235 rather than 2245. At page 12, lines 7 and 8, the beginning of the U3 region of the long terminal repeat is changed from nucleotide 7845 to nucleotide 7842. The support for this amendment can be found at page 3, line 24, and from Figure 1a.

Applicant has cancelled claims 2 to 26 and hereby presents claims 27 through 46. Applicant submits that the newly presented claims are supported throughout the specification, including at page 4, lines 32-37; page 6, lines 22-25; pages 9-11; and page 27.

Applicant hereby requests that the sequence listing entered in the parent case be entered into this continuation application.

Applicant requests entry and consideration of this Preliminary Amendment. Applicant requests that the examiner assigned to the case contact Applicant's representative if prosecution may be assisted.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

The paragraph beginning at page 3, line 8, has been amended as follows:

According to a first embodiment, the object of the invention is a recombinant retroviral vector for the cloning and/or express and/or transfer of an exogenous nucleotide sequence, characterized in that it consists of any sequence contained in the ClaI-PvuII fragment situated approximately between nucleotides 7702 and 1527 (SEQ ID NO: 13) of the sequence given in Figure 1 and comprising the LTR sequence included between nucleotides 7842 and 144, the PBS site starting at nucleotide 145, the packaging sequence included in the sequence of 250 nucleotides following the end of the LTR sequence, the said sequence being capable of controlling the cloning and/or expression and/or transfer of the exogenous sequence.

The paragraph beginning at page 3, line 19, has been amended as follows:

According to another embodiment of the invention, the recombinant vector is characterized in that it consists of any sequence contained in the ClaI-BamHI fragment comprising the nucleotides 7702 to 310 (SEQ ID NO: 13) of the sequence shown in Figure 1, and comprising the LTR sequence included between the nucleotides 7842 and 144, the PBS site starting at nucleotide 145, the packaging sequence included in the sequence of 250 nucleotides following the end of the LTR sequence, the said sequence being capable of controlling the cloning and/or expression and/or transfer of the exogenous sequence, whatever its transcriptional orientation with respect to the transcriptional orientation of the virus.

The paragraph beginning at page 3, line 30, has been amended as follows:

According to this second embodiment of the invention, the vector is thus a retroviral vector for the cloning and/or expression and/or transfer of an exogenous nucleotide sequence consisting of any sequence contained in the ClaI-BamHI fragment situated approximately between nucleotides 7702 and 310 (SEQ ID NO: 13) of the

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Preferably the recombinant vector additionally comprises a part of the gag sequence situated between the nucleotides 619 and [2245] 2235 (SEQ ID NO: 14) of the sequence shown in Figure 5, in particular the sequence included between nucleotides 619 and 1527 (SEQ ID NO: 15) of the sequence shown in Figure 1.

The paragraph beginning at page 6, line 21, has been amended as follows:

A useful vector of the invention lacking the envelope sequence [consists] consisting of the fragment comprising nucleotides 7806 to 1527 (SEQ ID NO: 12) of the sequence shown in Figure 1.

The paragraph beginning at page 6, line 24, has been amended as follows:

The invention also relates to a recombinant vector such that the sequence contained in the ClaI-PvuII fragment (7702-1527) (SEQ ID NO: 11) and/or this fragment and/or the sequence contained in the fragment ClaI-BamHI (7702-310) (SEQ ID NO: 13) and/or this fragment is replaced either by a sequence hybridizing under conditions of high stringency with the sequence corresponding to the above-mentioned fragments or by a sequence having an at least 95% nucleotide homology with the sequence corresponding to the above-mentioned fragments or at least 85% homology in the case of the U3 sequence.

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Other characteristics and advantages of the invention will become apparent in the examples and Figures which follow.

Figure 1: Sequence of viral DNA used for the construction of the vector FOCH29.

Figure 2: A: Restriction map of the vector FOCH29.

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U3 ----› R410 ([7845]7842-8255)
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2°) - for the second pair:

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5' GATCTGAACT TCTCTATTCTTG-3' (SEQ ID NO: 6)--

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The paragraph beginning at page 30, line 1, has been amended as follows:

In this perspective of improving viral safety, an alternative construction was prepared in which the GAG of FB29 is cut at the unique AhaIII (or isoDraI) site at position 1031 (SEQ ID NO:16); only a quarter of the GAG sequences are then conserved. The cut generated by AhaIII having blunt ends as for PvuII used for the cloning of FOCH29, the construction was made exactly superposable, the cloning upstream not being modified; downstream, the blunted ended SmaI site of the polylinker of pUC19 was used.